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10/658,602	09/08/2003	Enrique Zudaire Ubani	701779	6049
45733 7590 12/31/2008 LEYDIG, VOIT & MAYER, LTD. TWO PRUDENTIAL PLAZA, SUITE 4900 180 NORTH STETSON AVENUE CHICAGO, IL 60601-6731				
EXAMINER SIMS, JASON M				
ART UNIT		PAPER NUMBER		
1631				
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary

Application No.

10/658,602

Applicant(s)

ZUDAIRE UBANI ET AL.

Examiner

JASON M. SIMS

Art Unit

1631

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --
Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 21 May 2008.
- 2a) ☒ This action is **FINAL**. 2b) ☐ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 9, 12, 15-18, 20, 21, 23, 26-28, 85, 87, 90-99 and 103-117 is/are pending in the application.
- 4a) Of the above claim(s) _____ is/are withdrawn from consideration.
- 5) ☐ Claim(s) _____ is/are allowed.
- 6) ☒ Claim(s) 9, 12, 15-18, 20-21, 23, 26-28, 85, 87, 90-99, 103-117 is/are rejected.
- 7) ☐ Claim(s) _____ is/are objected to.
- 8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on _____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some * c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
 2. ☐ Certified copies of the priority documents have been received in Application No. _____.
 3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- 1) ☐ Notice of References Cited (PTO-892)
- 2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
- 3) ☐ Information Disclosure Statement(s) (PTO/SB/08)
Paper No(s)/Mail Date _____
- 4) ☐ Interview Summary (PTO-413)
Paper No(s)/Mail Date _____
- 5) ☐ Notice of Informal Patent Application
- 6) ☐ Other: _____

DETAILED ACTION

Applicant's arguments, filed 5/21/2008, have been fully considered. The following rejections and/or objections are either reiterated or newly applied. They constitute the complete set presently being applied to the instant application.

Applicants have amended their claims, filed 5/21/2008, and therefore rejections newly made in the instant office action have been necessitated by amendment.

Applicant has newly added claims 115-117 in the response filed 5/21/2008, which is acknowledged and been entered.

Applicant's cancellations of claims 11, 13-14, 22, 24-25, 86, 88-89, and 100-102 in the response filed 11/13/2007 is acknowledged.

Claims 9, 12, 15-18, 20-21, 23, 26-28, 85, 87, 90-99, 103-117 are the current claims hereby under examination.

Claim Objections

Response to Arguments:

Applicant's arguments, filed 5/21/2008, with respect to the objection of claims 106-108 have been fully considered and are persuasive because of applicant's arguments. Therefore the objection has been withdrawn.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 109-111 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 109-111 comprise the acronym CT, which has not been found to be a common acronym in the art and therefore has been found to be vague and indefinite. The acronym CT requires a definition in the claim language. Clarification via clearer claim wording is required.

The following rejection has been modified, which was necessitated by amendment:

Claim Rejections - 35 USC § 103-Modified

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

The factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

Claims 9, 12, 15-18, 20-21, 23, 26-28, 85, 87, 90-108, and 112-114 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer et al. (US P/N 6, 472,156) in view of Loffler et al. (US P/N 2003/0186259).

The claims are directed to a method of real-time detecting and quantifying a first nucleic acid template and a second nucleic acid template in a PCR mixture comprising the steps of thermally cycling a PCR mixture, obtaining first and second emissions, and determining the first and second emission amounts.

Wittwer et al. teaches limitations of claims 9, 21, 85, 97-99, and 103-108 at col. 1, lines 13-22, col. 2, lines 25-67 and col. 3, lines 1-37, col. 3, lines 40-67, col. 4, lines 1-18, lines 31-35, lines 42-47. Wittwer et al., at col. 1, discuss how the invention relates to multiplex PCR using differential fluorescent emission and differential hybridization melting temperatures, which allows simultaneous analysis. Wittwer et al., at col. 2 and col. 3, discusses step a of claims 9, 21, and 85 a thermal cycling method, using a thermostable polymerase, dyes that bind to double stranded DNA and multiple primers and probes for amplifying multiple sequences of DNA. Wittwer et al. further teach at col. 3, lines 4-7 and col. 17, lines 1-18 using a double stranded DNA intercalating dye. Wittwer et al. discuss, step b of claims 9, 21, and 85 at col. 3 and col. 4, using at least 2 probe pairs where one member of each pair differentially hybridizes to different alleles and measuring the emission of each of the members at a first temperature and repeating those emission measurements at a second and third temperature, which represents obtaining cycle by cycle at a first MT and a second MT the emissions and also reads on intermittently obtaining emission readings during each cycle. Wittwer et

al. further discuss part c of claims 9, 21, and 85 in col. 4 at lines 28-35 and 42-47, the different hybridizations having different emissions at different Tms and determining the different emissions based on the different Tms, which represents determining cycle by cycle a first emission amount and a second emission amount. Wittwer et al. at col. 3, lines 40-67 and col. 4 as discussed above teach taking emission readings at first and second temperatures through each thermocycle, which reads on the first emission and the second emission are the only emission readings of the double stranded intercalating dye obtained during each thermal cycle. Wittwer et al. at cols. 22-23 teach using controls for calibrating the emission and melting curves, which reads on using standards as in step e) wherein comparisons are made to obtain a calibrated curve.

Wittwer et al. teach limitations of claims 12, 23, and 87 at col. 3, lines 4-7.

Wittwer et al. discuss the background to PCR using nucleic acid binding dyes such as ethidium bromide and SYBR Green I, which are double stranded DNA intercalating dyes.

Wittwer et al. teach limitations of claim 24 at col. 11, lines 66-67 and col. 12, lines 1-5. Wittwer et al. discuss using a PCR primer as a "probe-primer," which represents the double stranded DNA dye as being a primer-based double stranded DNA dye.

Wittwer et al. teach limitations of claim 25 at col. 12, lines 15-20. Wittwer et al. discuss acceptable fluorophore pairs for use as fluorescein and rhodamine among others.

Wittwer et al. teach limitations of claims 15-18, 26-27, 90-94, and 96 at col. 15, lines 60-67 and col. 16, lines 1-45. Wittwer et al. discuss emission measurements being made every 50 to 10,000 msec and the temperature between measurements varying by 0.01 degrees Celsius per second to 5 degrees Celsius per sec or varying by 0.5 or 1.0 degrees Celsius per second. Wittwer et al. discuss how initial temperatures for initial emission measurements are made at low temperatures and subsequent emission measurements are made at higher temperatures until at least melting temperatures. Therefore the increased increments of temperature ranges ensures at least 3 or more emission measurements, which represents increasing the temperature at which emission measurements are taken in the markush ranges specified in the instant claims.

Wittwer et al. teach limitations of claims 20, 28, and 95 at col. 25, lines 65-67. Wittwer et al. discuss using the Lightcycler software for PCR and melting curve analysis, which represent a computer program for calculating first and second emissions.

Wittwer et al. suggest, but does not explicitly teach the limitation of quantifying the first and second amplicons as in step C of claims 9, 21, and 85. Wittwer et al. suggest this embodiment in the invention by stating at col. 11, lines 2-3 and col. 17, lines 20-23 the use of multiplexing and the use of hybridization probes as means for quantification. Therefore, Wittwer et al. recognize that quantifying the amplicons using the methods within the invention is possible and within the scope of one of ordinary skill in the art.

Loffler et al. specifically teach at paragraph [0014] that the extent of fluorescence during FRET is directly proportional to the amount of target DNA, which is generated during the PCR process. Loffler et al. teach that as a result of this, the increase in DNA produced can be monitored via an increase in the fluorescence signal. Furthermore, Loffler et al. at paragraph [0039] teach that the emission being detected not only identifies the amplicon, but also enables quantification of the amplicon in the reaction solution.

It would have been obvious to one of ordinary skill in the art at the time of the instant invention to quantify the amplicons during each cycle in the PCR reaction because Wittwer et al. states that the methodology of using the hybridizable probes in FRET can be used for quantification and Loffler et al. specifically teaches how quantification is done using hybridizable probes in FRET analysis.

Wittwer et al. do not specifically teach wherein the first and second amplicons have melting curves, which do not overlap as in claims 100-102.

However, Wittwer et al. do teach being able to analyze multiple sequence samples simultaneously and at Fig. 1 gives examples of the different melting curves for three samples. It would have been obvious to one of ordinary skill in the art at the time to apply the known method taught by Wittwer et al. to samples or amplicons, which do not overlap in their melting curves because analyzing samples or amplicons without overlapping melting curves would have been a simple substitution of some components. It is well known that different sequences have different melting curves as discussed throughout the invention by Wittwer et al. col. 17, lines 1-18. Therefore, it is obvious to

one of ordinary skill in the art at the time of the instant invention that one could have substituted one known element, which are amplicons with melting curves that do not overlap, with the known method for performing hybridization analysis and the results would have been predictable.

With regards to claims 112-114: Wittwer et al. do not teach wherein the primers are covalently linked to a dye.

Claims 115-117 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer et al. (US P/N 6, 472,156) in view of Loffler et al. (US P/N 2003/0186259) as applied to claims 9,21, and 85 above, and further in view of Wittwer et al. and Loffler et al.

With regards to claims 115-117: Wittwer et al. at col. 17, lines 46-65 teach using a program for calibrating the emission vs temperature curves.

Wittwer et al. do not explicitly teach a mathematical relationship between the emission reading and the quantification of the amplicon.

Loffler et al. at paragraphs [0014] and [0039] teach that there is a mathematical relationship between emission readings and quantification of amplicons.

It would have been obvious to one of ordinary skill in the art at the time of the instant invention to have used a computer program as taught by Wittwer to quantify amplicons based on emission readings as taught by Loffler et al. This is because automating, i.e. using computers and computer programs, is an obvious application of a

known improvement technique to a base method wherein the results would have been predictable and more accurate to one of ordinary skill in the art.

Response to Arguments:

Applicant's arguments filed 5/21/2008 have been fully considered but they are not persuasive.

Applicant argues that Wittwer does not teach or suggest the quantification of first and second amplicons, wherein the melting curves of the first and second amplicons do not overlap. Applicant further alleges that it is not possible for the Wittwer reference to quantify first and second amplicons simultaneously as claimed because the first amplicon will be giving a first emission reading at the same time that the second amplicon is giving a second emission reading. Applicant further states that emission readings happening at the same time would cause the signal of the two amplicons to be mixed up without being able to quantify them separately.

Applicant's arguments are not found persuasive because Wittwer at col. 1, lines 20-23 teaches that the invention allows simultaneous analysis of three or more fluorescent labels having different emission spectra, which reads on enabling the simultaneous analysis of two different amplicons labeled with different fluorescent labels, wherein the labels have different emission spectra. Furthermore, Wittwer teaches at Fig. 17 a wherein different amplicons display melting curves which do not overlap by much yet the different amplicons are able to be detected. Therefore, to detect and quantify amplicons without overlapping melting curves would be determined

by routine optimization methods wherein it would be obvious that detection and quantification of amplicons without overlapping melting curves would improve the reliability. Furthermore, Wittwer at col. 11, lines 2-6 teach that multiplexing satisfies a need for quantification wherein multiple amplicons can be analyzed, i.e. quantified, in a single reaction mixture. Wittwer further teaches at col. 11, lines 20-21 taking emission readings at different increasing temperatures.

Applicant further argues that in order to quantify the amplicons, emission readings must be obtained during each thermal cycle of the PCR protocol, wherein Wittwer does not obtain emission readings during each thermal cycle of the PCR. Applicant further argues that because of this it is not possible for the method of Wittwer to quantify first and second amplicons.

Applicant's arguments are not found persuasive because Wittwer at col. 11, lines 2-6 teach that multiplexing satisfies a need for quantification wherein multiple amplicons can be analyzed, i.e. quantified, in a single reaction mixture. Furthermore, Wittwer teaches at col. 11, lines 20-21 taking emission readings at different increasing temperatures. Therefore Wittwer discusses a method wherein quantification of multiple amplicons from the same reaction mixture is possible. Frequency of cycling is a result optimized parameter normally determined by routine optimization and if the reference method could successfully operate at less frequent cycling it would be obvious it would perform with increase reliability at an increased frequency, i.e. taking emission readings at every cycle of the PCR protocol.

Applicant further argues that Wittwer does not teach non-overlapping melting curves or measuring emission amounts during each thermal cycle. Applicant then further argues that Wittwer teaches nothing about non-overlapping melting curves of multiple amplicons to be quantified in a single PCR reaction.

Applicant's arguments are not found persuasive as applicant uses an emission vs cycle curve to quantify the amplicons and not the melting curves, i.e. as recited in claim 9, steps c) and d) and therefore arguments are not commensurate in scope with the claimed invention. Furthermore, Wittwer at col. 17, lines 1-4 teaches monitoring emissions continuously through repeated cycles during PCR. Wittwer further teaches at col. 17, lines 19-22 that the melting of hybridization probes can be continuously monitored, wherein probe melting characteristics can be exploited for product quantification.

Appliant argues that Wittwer does not teach obtaining a standard emission curve and comparing the emission readings with the standard.

Applicant's arguments are not found persuasive because Wittwer at cols. 22-23 teaches using controls for calibrating the emission and melting curves, which reads on using standards as in step e) wherein comparisons are made to obtain a calibrated curve.

Applicant argues that Loeffler fails to cure the deficiencies of Wittwer.

Applicant's arguments are not found persuasive as Wittwer teaches that multiplexing can be used for quantification, multiple amplicons can be analyzed in one reaction mixture, and monitoring emission readings during each cycle. Loeffler taught at

paragraph [0014] that the extent of fluorescence during FRET is directly proportional to the amount of target DNA, which is generated during the PCR process. Loffler et al. teaches that as a result of this, the increase in DNA produced can be monitored via an increase in the fluorescence signal. Furthermore, Loffler et al. at paragraph [0039] teaches that the emission being detected not only identifies the amplicon, but also enables quantification of the amplicon in the reaction solution.

Conclusion

No claim is allowed.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire **THREE MONTHS** from the mailing date of this action. In the event a first reply is filed within **TWO MONTHS** of the mailing date of this final action and the advisory action is not mailed until after the end of the **THREE-MONTH** shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than **SIX MONTHS** from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Jason Sims, whose telephone number is (571)-272-7540.

If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's supervisor, Marjorie Moran can be reached via telephone (571)-272-0720.

Papers related to this application may be submitted to Technical Center 1600 by facsimile transmission. Papers should be faxed to Technical Center 1600 via the Central PTO Fax Center. The faxing of such papers must conform with the notices published in the Official Gazette, 1096 OG 30 (November 15, 1988), 1156 OG 61 (November 16, 1993), and 1157 OG 94 (December 28, 1993) (See 37 CFR § 1.6(d)). The Central PTO Fax Center number is (571)-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

// Jason Sims //

/Michael Borin/
Primary Examiner, Art Unit 1631